CHRONIC HEALTH EFFECTS TESTING FOR HYDROQUINONE

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[+] *The Nonprescription Drug Manufacturers Association (NDMA) is a 111-year-old U. S. trade organization representing the manufacturers and distributors of nonprescription (or over-the-counter) medicines.*

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I. Executive Summary

From an in-depth review of the entire database on hydroquinone, the Nonprescription Drug Manufacturers Association (NDMA) Hydroquinone (HQ) Task Group concludes that hydroquinone in OTC skin lightening preparations does not present a human carcinogenic risk when used according to label directions. This conclusion is based on differences in species- and strain-specific mechanisms of toxicity which contribute to the animal bioassay results and an evaluation of the genotoxicity database.

For several years, hydroquinone (HQ) has been the subject of a series of chronic health effects studies under the testing programs of the U.S. Environmental Protection Agency (see the Toxic Substance Control Act Section 4) and the U.S. National Toxicology Program. These studies have included endpoints for neurotoxicity, reproductive toxicity, developmental toxicity, genotoxicity, pharmacokinetics, chronic toxicity, and carcinogenesis. The results of these studies and many more in the published literature have created a data base for HQ that is much more extensive than is available for many other chemicals.

Much of these data are derived from studies designed to maximize the likelihood of producing a toxic response by administering HQ in a high-dose bolus by the gavage route. While on the one hand such studies may be well designed for hazard identification, they have the potential for producing misleading results for risk assessment purposes. This is particularly the case for chemicals such as HQ where the primary route of exposure is dermal (not oral, as in the case of the animal bioassays) and where dermal absorption does not result in significant toxicity.

In such circumstances, the results of oral or parenteral studies should be considered crude estimates of effects. Such studies are adequate for preliminary hazard identification, which can often -- and should, where possible -- be refined by additional research, particularly involving studies by the dermal route.

Furthermore, the situation with HQ is more complex than simply trying to extrapolate data from studies using an inappropriate route (oral) to a relevant route (dermal), since toxicity related to specific species or strains can affect interpretation of the observations. Thus, the results of the recent animal bioassays on HQ should not be extrapolated directly for human risk assessment purposes because the tumor endpoint of concern (kidney adenomas) appears to be related to a species-specific toxicity particular to the male F-344 rat.

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The other genotoxicity assays conducted with HQ should not be interpreted to indicate that HQ is likely to present a genotoxic risk to humans or be used to support the idea that HQ exposure results in tumorigenicity. This is because none of the assays are designed to be directly extrapolated for human risk assessment purposes. These in vitro studies on HQ greatly exaggerate the risks associated with HQ exposure, and HQ has produced negative results in Ames/Salmonella and Drospophila assays conducted by routine procedures. Chromosomal effects, particularly those relating to micronuclei, do not predict bioassay results in the target tissue (bone marrow) or the target species (mouse) and therefore can not be used to predict effects in humans or other animal species.

Finally, a substantial amount of research is now underway including additional studies designed to better understand the mechanistic issues related to the species and strain specific toxicity that HQ possesses.

Notwithstanding the ongoing research program, a review of the entire HQ database, with appropriate weight given to mechanistic data on species and strain-specificity of the carcinogenic response, yields a conclusion that HQ in nonprescription skin lightening creams does not present a human carcinogenic risk when used according to label directions.

The sections of this report are organized as follows:

II. Index

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III. Results Following Dermal Application

A. HQ Percutaneous Absorption is Vehicle Dependent

Percutaneous absorption of HQ in aqueous solutions is poor. Alcoholic solutions may increase the penetration of HQ, but even under these conditions, HQ elimination from the body is rapid and, therefore, HQ body burdens remain low. Commercial OTC preparations contain only aqueous vehicles -- not alcoholic vehicles, which are known to enhance the absorption of HQ. As such, HQ absorption from straight aqueous vehicles would be expected to be less than that from experimental alcoholic vehicles.

The percutaneous penetration of HQ has been reported in three studies: an in vivo human assessment; an evaluation using excised human skin and skin from hairless mice in vitro; and an in vivo study in rats.

Bucks et al.[3] (1988) applied 14C-labeled HQ in an alcoholic vehicle[1] to the forehead skin of male human volunteers and measured HQ excretion in the urine. Using different formulations containing 2% HQ in alcoholic vehicles -- including other ingredients known to enhance absorption -- varying amounts of the applied dose were absorbed in 24 hours. Curiously, the skin penetrant enhancer, 1-2dodecylazacycloheptan-2-one, did not affect the penetration of HQ, while the addition of 2-ethylhexyl ester of 4-(dimethylamino) benzoic acid significantly decreased absorption.

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[1] Note: Commercial OTC preparations contain only aqueous vehicles -- not alcoholic vehicles. As such, HQ absorption from OTC aqueous vehicles would be expected to be less than that from experimental alcoholic vehicles. See also Marty et al. (1981) and English et al. (1988).

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In contradistinction to the results of Bucks et al.[3], Marty et al.[34] (1981) studied the in vitro and in vivo penetration of HQ in an oil/water emulsion through the skin of hairless rats and in vitro through human skin. Under these conditions, HQ was poorly absorbed through rat skin and absorption was seven times slower through human skin than through rat skin.

Similarly, English et al.[13] (1988) found that 14C-labelled HQ was poorly absorbed through the skin of F-344 rats in vivo from an aqueous solution applied to the skin for 24 hours.

The foregoing indicate that the percutaneous absorption of HQ in ordinary aqueous solutions is low, and that alcohol -- but not azone or the sunscreen 2-ethylhexyl ester of 4-(dimethylamino)benzoic acid -- can increase the penetration of HQ. But, even under these conditions, HQ elimination from the body is rapid and, therefore, HQ body burdens remain low.

B. Toxicity Following Dermal Application of HQ is Unremarkable

The majority of studies of HQ following dermal application are unremarkable in terms of the nature and extent of potential toxicity from HQ.

Burnett et al.[4] (1976) studied the percutaneous toxicity (i.e., twice weekly dermal application to rabbits for 13 weeks) and teratogenicity (i.e., dermal application to pregnant rats on 1, 4, 7, 10, 13, 16, and 19 days of gestation) of a hair dye containing 0.2% HQ and found no evidence of toxicity. Similarly CTFA commissioned dermal teratology studies (i.e., dermal application from day 6 through day 19 of gestation in pregnant rats) of HQ and a skin lightener containing HQ which did not show evidence of developmental toxicity (see CTFA,[10] 1980).

NTP[39] (1989) conducted a 14-day dermal toxicity study using groups of five rats or mice per sex. Rats were given up to 3.8 g/kg of HQ in 95% ethanol and mice received up to 4.8 g/kg HQ five days per week for 12 doses over 14 days. Even though HQ was qualitatively detected in the urine of male rats exposed to HQ, no evidence of toxic effects was seen.

An exception is the report of Sivchev et al.[52] (1966) in which guinea pigs were covered (90% of body surface) with a vaseline ointment containing up to 20% HQ daily for 50 days. The 20% HQ ointment provided approximately 500 mg of applied

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HQ per day. The application areas were not covered, and it therefore is noteworthy that the guinea pigs apparently were free to ingest the ointment. Under these conditions, histologic changes were reported in the liver, kidneys, heart, spleen, lungs, and adrenal glands and hematologic changes were found. In retrospect, a number of the lesions observed may have been due to intercurrent infectious disease, as evidenced histopathologically by signs of pneumonia in three animals and catarrhal inflammation of the bronchi and bronchioles in the remaining animals.

C. HQ Has Been Reported to Have Inhibitory Activity on B[a]P-induced Carcinogenesis and Promotes Survival of Animals With Transplanted Melanoma

In a screening study for co-carcinogenesis and tumor promoting agents in tobacco, Van Duuren and Goldschmidt[57] (1976; **Table 1**) found that HQ did not induce tumors after skin application and was a weak inhibitor of carcinogenesis initiated by benzo[a]pyrene (B[a]P). These studies were designed with groups of 50 female CR/HA Swiss mice given acetone, 5 mg HQ in acetone, or 5 mg HQ in acetone preceded by 5 ug B[a]P three times per week or 150 ug B[a]P once two weeks prior to HQ administration. HQ was administered three times per week for 52-58 weeks. Animals given HQ in acetone developed no tumors, while those given B[a]P and HQ developed fewer neoplasms (7 of 11 mice) than mice given only B[a]P (14 of 16 mice).

HQ has also shown a moderate protective effect against a common skin tumor (melanoma) transplanted into mice. Mice with tumor transplants given 80 mg/kg HQ subcutaneously for nine days showed survival times which were equivalent to those mice not receiving transplants (Chavin et al.,[66] 1980).

Table 1

HQ Dermal Bioassay in CR/HA Swiss Mice w/wo Initiator

No./Fmls/Grp:	50	50	50
Initiator:	None	5µg Benzo[a]pyrene 3x/week	150µg Benzo[a]pyrene 1x/2wk prior to HQ
Vehicle:	Acetone	Acetone	Acetone
HQ Dose Levels:	5 mg	5 mg	5 mg
HQ Dose Freq.:	3x/week	3x/week	3x/week
Study Length: (weeks)	52	52	58
Results:	No tumors	HQ Weak Inhibitor 3 SQ Cell-HQ/BaP 10 SQ Cell-BaP	HQ Weak Inhibitor

Data from: VanDuuren and Goldschmidt (1976)

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IV. Oral Bioassay Results: An Issue of Species- and Strain-Specificity

A. Introduction

There are three HQ bioassays which have been conducted by oral administration. The protocols and results are outlined in **Tables 2a and b on page 8.**

In the earliest of these studies (Carlson and Brewer,[5] 1953), HQ was incorporated into diets which were fed to Sprague-Dawley rats seven days per week for 104 weeks. Carcinogenicity was not observed in any organ system **(Table 2a and b).**

NTP[39] (1989; **Tables 2a and b**) conducted a chronic bioassay in F-344 rats and B6C3F mice given HQ by gavage (25 or 50 mg/kg rats and 50 or 100 mg/kg mice). In this study, renal toxicity was observed in male and female rats; renal adenomas were observed in male rats; a trend towards a higher incidence in mononuclear cell leukemia was present in female rats; and an increase in liver tumors (adenomas and carcinomas combined) was seen in female mice. The liver tumor increase in female mice was due to an increase in adenomas. In male mice, a higher incidence of liver adenomas was also seen, but because there was a decreased incidence of malignant liver tumors, the combined benign and malignant tumor incidence was not considered significant. NTP classified these responses as "some evidence" of carcinogenicity.

Shibata et al.[50] (1991; **Tables 2a and b**) gave F-344 rats and B6C3F mice diets containing 0.8% HQ. This diet provided dose levels of approximately 360 mg/kg (rats) and approximately 1300 mg/kg (mice). In this study (Table 2), renal toxicity in male and female rats and kidney tumors in male rats were observed. No mononuclear cell leukemia was observed in female rats. In mice, there was an increase in benign liver tumors only in male mice. Renal toxicity in the form of renal hyperplasia was observed in male mice, and there was a statistically insignificant increase in benign kidney tumors in male mice as well. The full range of renal pathology changes was not observed in this study as there were no interim sacrifices to collect renal tissue for histopathology.

B. Qualitative Assessment of the Oral HQ Bioassay Results

Extrapolation of animal bioassay data should involve a broad ranging assessment of all of the data available on a chemical. Risk assessments are sometimes based on data derived from experiments conducted by irrelevant routes of exposure (e.g., use of the

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Table 2a

HQ Two-Year Bioassay (Oral Routes) - 1

		Carlson and Brewer (1953)	NTP (1989)	Shibata *et al.* (1991)
Species:		S-D Rats	F344 Rats/ B6C3F$_1$ Mice	F344 Rats/ B6C3F$_1$ Mice
No/Sex/Grp:		46-53[1]	55	30
Route:		0.1, 0.25, 0.5, 1.0% HQ Diets	Gavage	0.8% HQ Diet
Dose Levels: (mg/kg/day)	Rats	~ 80-800	25 & 50	~ 360
	Mice		50 & 100	~ 1300
Dose Freq.: (day/week)		7	5	7
Study Length: (weeks)	Rats	104	103	104
	Mice		103	96

[1] The 0.25% Group was 16-23 rats/sex/grp. Some rats were used for interim studies. Exposures included HQ (10 rats/sex/grp); HQ + 0.1% citric acid (16-23 rats/sex/grp); and HQ mixed in lard and heated to 190°C for 30 min (16-23 rats/sex/grp).

Table 2b

HQ Two-Year Bioassay (Oral Routes) - 2

Carlson and Brewer (1953)	NTP (1989)	Shibata *et al.* (1991)
Rats		
No	Kidney Toxicity - M/F	Kidney Toxicity - M/F
No	Kidney Adenomas - M	Kidney Adenomas - M
No	Mononuclear Cell Leukemia - F	No
Mice		
	No	Kidney Hyperplasia - M
	No	Kidney Adenomas - M (NS)
	Liver Adenomas - M (NS)[1]	Liver Adenomas - M
	Liver Adenomas - F	No

[1] Not significant when combined with carcinomas.

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oral route to assess risk of human dermal exposure without any regard to route-specific pharmacokinetics) and using inappropriate animal model systems.

Both of these errors could potentially be made in the assessment of the HQ bioassays.

On a qualitative basis, the following statements can be made about the HQ oral bioassay results. These points are also discussed further in Sections C, D, and E below.

- Renal adenomas in male F-344 rats are associated with an interaction between nephrotoxicity and chronic nephropathy which may be unique to the male F-344 rat.

- Renal adenomas in male mice are associated with large oral exposures to HQ which are likely to overwhelm normal metabolic pathways resulting in nephrotoxicity.

- Mononuclear cell leukemia in female rats is not a reproducible effect indicative of carcinogenicity.

- The incidence of hepatocellular adenomas in B6C3F mice is questionable -- and certainly not "some" or "definite" -- evidence of carcinogenicity.

C. HQ Exposure is Associated with Significant Renal Toxicity Only in the F-344 Rat

In reviewing the HQ data base, there are several studies which have assessed the effects of HQ on renal structure or function. The endpoints that were used for this review included alterations in kidney weight, renal histopathology, and urinalysis.

For the F-344 rat, there are four studies which assessed renal effects associated with subchronic or chronic HQ exposure to male or female rats (NTP,[39] 1989 and Shibata et al.,[50] 1991). The protocols and results of these studies are outlined in **Table 3**. The results of these studies consistently show that HQ alters renal structure in the F-344 rat and that subchronic and chronic HQ exposure affects the male F-344 rat more severely than the female (**Table 3**).

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Table 3

HQ Renal Toxicity - F-344 Rats

	NTP (1989)	NTP (1989)	NTP (1989)	Shibata *et al.* (1991)
No./sex/grp.:	10	10	55	30
Route:	Gavage in corn oil	Gavage in water	Gavage in water	0.8% HQ in diet
Dose Levels: (mg/kg/day)	25, 50, 100, 200, 400	25, 50	25, 50	~ 360
Dose Freq.: (days/week)	5	5	5	7
Study Length: (weeks)	13	65	103	104
Kidney weight:	ND	Inc. % BWT 50 mg/kg M	Inc. % BWT 50 mg/kg M	Inc. Abs. & % BWT - M Inc. % BWT - F
Urinalysis:	ND	ND	ND	ND
Histopathology: (nephropathy)	200 mg/kg: 7/10 M 6/10 F 100 mg/kg: 1/10 F	Inc. Severity 50 mg/kg: 6/10 M 25 mg/kg: 5/5 M	Inc. Severity 50 mg/kg: 20/55 M	Inc. Severity M: 14/30 F: 7/30 (minimal)

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Table 4 outlines the protocols and results of four studies with HQ exposure to Sprague-Dawley (Topping et al.,[54] 1988; Carlson and Brewer,[5] 1953) or Carworth rats (Christian et al.,[7] 1976; two studies). The most significant effect on the kidney was slight increases in relative kidney weight (%BWT). In none of these studies was there evidence of structural damage to the kidney.

Tables 5 and 6 outline the protocols and results used to study HQ effects in dogs and humans (Carlson and Brewer,[5] 1953). While both of these studies involved relatively small number of subjects, the dose levels, exposure periods, and detection methods should be considered adequate to detect nephrotoxicity. None was detected. The 300 mg/day dose level given to humans equates to approximately 4 mg/kg/day on a body weight basis and is equivalent to a rodent dose of approximately 25 mg/kg/day on a body surface area basis.

Based on a comparison of the results of these studies, it is clear that F-344 rats develop nephrotoxicity following HQ exposure and Sprague-Dawley and Carworth rats do not (**Tables 3 and 4**). The results of the dog studies, in which no toxicity was found, support the conclusion that the F-344 rat has a unique susceptibility to HQ. For humans, it is clear that significant subchronic exposure does not result in nephrotoxicity (Carlson and Brewer,[5] 1953). Carlson and Brewer[5] (1953) concluded:

> "Man. Analyses of the urine and blood of 19 human subjects that ingested 300 to 500 mg of hydroquinone daily for 3 to 5 months revealed no abnormal findings." (Carlson and Brewer,[5] 1953 at page 686; emphasis supplied)

This conclusion is supported by an epidemiology study conducted by Friedlander et al.[16] (1982) on employees of a photographic processor where HQ exposures were low (< 0.01 mg per cubic meter) and "no significant excess mortality, sickness absence or cancer incidence" were noted. Additional epidemiologic support is provided by a case report study of ocular toxicity among employees of a hydroquinone manufacturing plant (Sterner et al.,[53] 1947 and Anderson,[68] 1947) whereby no systemic toxicity was observed under conditions which led to corneal pigmentation. Sterner et al.[53] observed:

> "Repeated physical examinations and laboratory studies did not reveal any evidence of systemic injury and the degree of dis-

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Table 4

HQ Renal Toxicity - S-D and Carworth Rats

	Topping *et al.* (1988)	Carlson and Brewer (1953)	Christian *et al.* (1976)	Christian *et al.* (1976)
Strain:	S-D	S-D	Carworth	Carworth
No./sex/grp.:	10	46-53[1]	6	20
Route:	Gavage in water	0.1, 0.25, 0.5, 1.0% HQ Diets	2.5, 5, 10 g/L in water	1, 2, 4 g/L in water
Dose Levels: (mg/kg/day)	20, 64, 200	~ 80-800	230 - 810	110- 430
Dose Freq.: (days/week)	5	7	7	7
Study Length: (weeks)	13	104	8	15
Kidney weight:	No effect	ND	Inc. @ 5 and 10 g/L	Inc. Rel. Wt. 1, 2, 4 g/L (1 g/L F NS)
Urinalysis:	ND	ND	ND	ND
Histopathology:	No effect	No effect	No effect	No effect

[1] The 0.25% Group was 16-23 rats/sex/grp. Some rats were used for interim studies. Exposures included HQ (10 rats/sex/grp); HQ + 0.1% citric acid (16-23 rats/sex/grp); and HQ mixed in lard and heated to 190°C for 30 min (16-23 rats/sex/grp).

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Table 5

HQ Renal Toxicity - Mixed-Breed Dogs

No./Dogs/Grp.:	1	2	5 males
Route:	Oral Tablet	Oral Tablet	Oral Tablet
Dose Levels: (mg/kg/day)	16	1.6 and 40	100
Dose Freq.: (days/week)	7	7	7
Study Length: (weeks)	80	1.6 mg/kg for 31 wks +40 mg/kg for 49 wks	26
Kidney Weight:	ND	ND	ND
Urinalysis:	No effect	No effect	No effect
Histopathology:	No effect	No effect	No effect

Data from Carlson and Brewer, 1953

Table 6

HQ Renal Toxicity - Human

Subjects:	2 males	17 males/females
Route:	Oral 3 divided doses with meal	Same
Dose Levels: (mg/day)	500	300
Dose Freq.: (days/week)	7	7
Study Length: (weeks)	20	12-20
Urinalysis:	No effect	No effect

Data from Carlson and Brewer, 1953

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> comfort sustained was almost entirely the limiting factor in controlling the quinone vapors until recognition of the above local eye injury."
>
> "Following the identification of the eye effects with the quinone-hydroquinone exposures, examination of the exposed workers for evidence of systemic absorption and intoxication was intensified, but no injury other than the local eye effects has been found. Complete physical examinations and comprehensive laboratory studies, including urine inorganic-total sulfate ratios have been made serially, and have been uniformly negative." (Sterner et al.,[53] 1947 at page 67; emphasis supplied)
>
> "The results of all of these characteristics studied [i.e., values for hemoglobin, leukocyte count, erythrocyte count, cell volume, sedimentation rate, polymorphonuclear count, icterus index, 'stab' cell count, lymphocyte count, eosinophile count, monocyte count, basophile count] show no significant deviations from those of the control group." (see Tables 1-12 of Sterner et al.,[53] 1947 at page 67-70; emphasis supplied)

Anderson [68] (1947) described the medical condition of this population as well:

> "Two of our patients (cases 8 and 41) have been hospitalized and subjected to exhaustive tests. Urinalysis and studies of the blood have been performed repeatedly on all persons employed in the plant. No suggestion of systemic intoxication with the drug, which after all is relatively harmless, has been observed." (Anderson [68], 1947 at page 11; emphasis supplied)

In summary, the studies by NTP, Shibata et al., Topping et al., Carlson and Brewer, Christian et al., Sterner et al., and Anderson demonstrate defferences in sex, strain, and species specificity. The mechanistic reason(s) for the differences in sex, strain, and species susceptibility are not completely known, but are being vigorously investigated. Boatman et al.[69] (1992) have developed an acute model for HQ toxicity that should allow for mechanistic studies on the role of various enzyme systems in the production of HQ toxicity. Boatman et al.[69] (1992) found that administration of 400 mg/kg HQ to either male or female F344 rats resulted in significant increased excretion of alanine aminopeptidase, n-acetyl glucosaminidase, alkaline phosphatase,

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gamma-glutamyl transpeptidase, and glucose which are markers of proximal renal tubular damage. Also significantly, Sprague-Dawley rats given the same dose levels of HQ did not increase their excretion of these marker substances. However, Boatman et al.[69] (1992) were able to induce nephrotoxicity in both F344 and Sprague-Dawley rats by intravenous injection of a hydroquinone conjugate.

Hill et al.[70] (1992) and Kleiner et al.[71] (1992) recently reported on the *in vivo* and *in vitro* formation of several conjugates of HQ in the Sprague-Dawley rat. They were also able to induce nephrotoxicity in Sprague-Dawley rats by intraperitoneial or intra-arterial (renal artery) injection of HQ conjugates.

These data suggest that the strain difference in susceptibility to HQ may not reside in the kidney since both F344 and Sprague-Dawley male rat kidneys respond to the HQ conjugate (**Table 7**). The most likely explanation for the difference in strain responses is that there is a significant difference in hepatic P450 metabolism that results in differences in the amount of glutathione conjugate formed, in the rate of glutathione conjugate formed, or the type of glutathione conjugate formed.

Table 7

HQ Renal Toxicity

Test System		Nephropathy	Urinalysis
F-44 Rats	Males	++	ND
	Females	+	ND
S-D Rats		-	ND
Carworth Rats		-	ND
Mixed-Breed Dogs		-	ND
Human		ND	No Effect

(continued . . .)

Similarly, the sex difference in HQ nephrotoxicity may be dependent on the differences in hepatic metabolism of HQ or the rate of delivery of HQ metabolites to the kidneys.

1. Renal Tumors F-344 Male Rats Exposed to HQ are Associated with Significant Renal Toxicity and Chronic Progressive Nephropathy

In both the study by Shibata et al.[50] (1991) and that by NTP[39] (1989), chronic HQ exposure was associated with an increased severity of chronic nephropathy in male F-344 rats. This relationship is shown in Table 8 which is modified from a table in the NTP report to also show the strong correlation between the occurrence of adenomas and the severity of nephropathy. Nine of the twelve tumors were observed in rats with end stage renal disease. This strong association with chronic nephropathy, which is recognized as a life limiting disease in male rats, may explain why female F-344 rats did not show an increased incidence of renal tumor, even through they share a susceptibility to nephrotoxicity with the male rats.

Table 8

Number of Male Rats with Indicated Severity of Nephropathy (Adenomas) in the NTP Two-Year Gavage Study of Hydroquinone

Severity	Control	25 mg/kg	50 mg/kg
No. of rats examined	55	55	55
No nephropathy	2	3	0
Minimal	3	1	3
Mild	12	12 (1)	5
Moderate	26	31 (1)	15 (1)
Marked	12	8 (2)	32 (7)
Total No. Adenomas	(0)	(4)	(8)

Historical Control Incidence Range 0-6%

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Recently collected data on repetitive dosing of male and female F344 rats provides insight into this strain difference (English, unpublished data). English gave male and female F344 rats 25 or 50 mg/kg/day (5 d/wk) of HQ by gavage for 1, 3, or 6 weeks and found that at six weeks there was an increase in the excretion of urinary enzymes and glucose only in male rats (50 mg/kg) and that the increased excretion of these materials was associated with an increase in the rate of proximal renal tubular cell proliferation.

During the NTP HQ bioassay, there was a concern that sex difference in the kidney adenoma response may have been due to a mechanism involving the renal accumulation of α-2u-globulin in the male rats. Therefore, the report specifically made mention that hyalin droplets were not observed in male rats during the HQ bioassay. Yet in many ways the pathogenesis of the tumors following HQ administration and in the case of α-2u-globulin may be very similar in that both cases the test materials induce cell toxicity and cell proliferation very early (between 3-6 weeks with HQ) after the initiation of dosing and in both cases the cell proliferation associated with chronic progressive nephropathy may contribute to the latter appearance of cell hyperplasia and benign tumors (Short and Swenberg,[72] 1991). **Figures 1A and 1B** outline the potential interaction between these simultaneous stimulators of cell proliferation.

(Figures 1A and 1B follow on pages 18 and 19; Text continues on page 20.)

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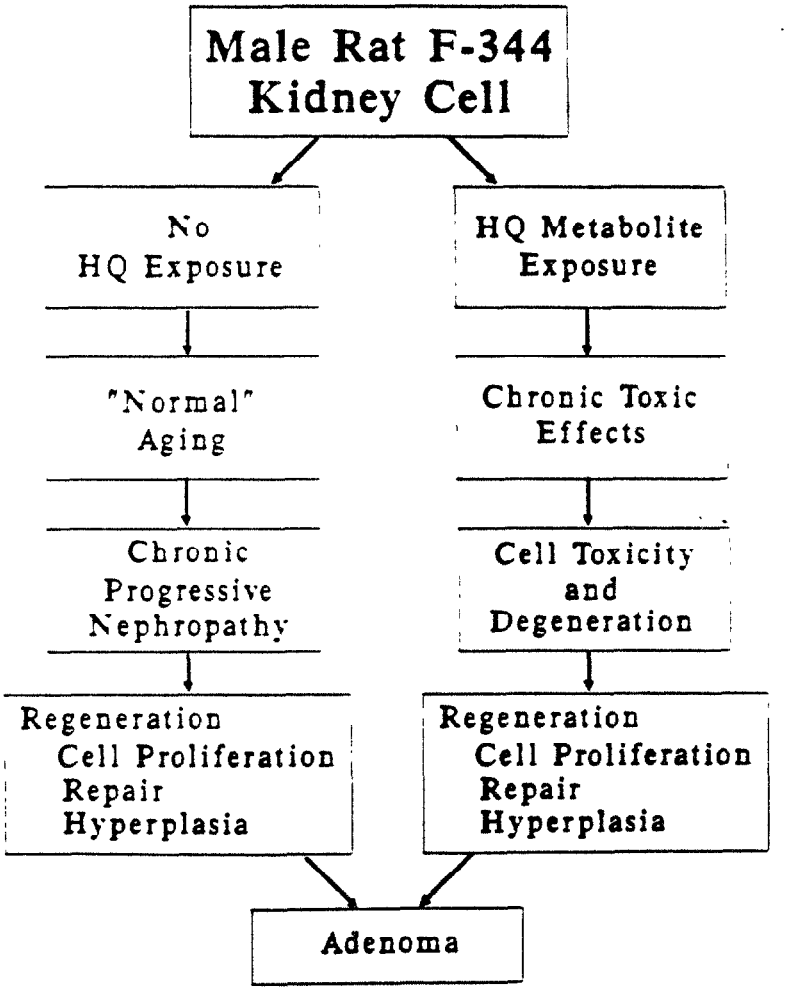
Figure 1A

Proposed Interaction Between CPN-induced and HQ-induced Cell Hyperplasia in the Male F-344 Rat



Similar interactions are not expected to occur in female F-344 rats, SD rats, or other species.

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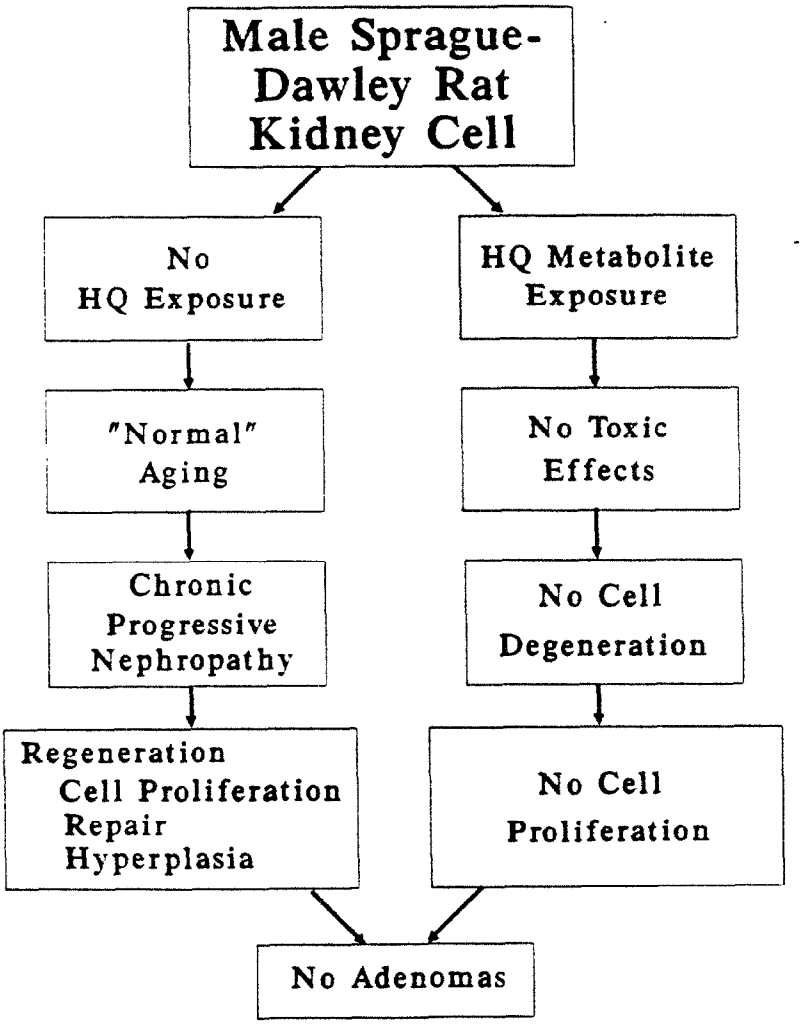
Figure 1B

Hydroquinone Exposure Does Not Result In Kidney Toxicity or Adenomas In Male Sprague-Dawley Rats



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2. **Renal Adenomas in Male Mice Are Associated With Large Oral Exposures to HQ Which Are Likely to Overwhelm Normal Metabolic Pathways Resulting in Nephrotoxicity**

Table 9 outlines the protocols and the results of the NTP[39] (1989) and the Shibata et al.[50] (1991) studies using B6C3F mice. In the NTP study, a transitory increase in relative kidney weight (% BWT) was observed only in female mice at 65 weeks of exposure. In contrast, Shibata et al.[50] (1991) reported finding renal tubular hyperplasia in 9/30 mice and adenomas in 3/30 mice. The latter finding was not considered statistically significant.

Table 9

HQ Renal Toxicity - B6C3F$_1$ Mice

	NTP (1989)	NTP (1989)	NTP (1989)	Shibata *et al.* (1991)
No./sex/grp.:	10	10	55	30
Route:	Gavage in corn oil	Gavage in water	Gavage in water	0.8% HQ in diet
Dose Levels: (mg/kg/day)	25, 50, 100, 200, 400	50, 100	50, 100	~ 1300
Dose Freq.: (days/week)	5	5	5	7
Study Length: (weeks)	13	65	103	96
Kidney weight:	ND	Inc. % BWT 50 and 100 mg/kg F	No effect	Inc. % BWT Females only
Urinalysis:	ND	ND	ND	ND
Histopathology:	No effect	No effect	No effect	Tubular Hyperplasia in 9/30 males

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The likely explanation for the difference in results between the two studies is that the dose level in the Shibata et al.[50] (1991) study (approximately 1300 mg/kg/day) overwhelmed the normal detoxification process in the liver resulting in non-linearity in HQ pharmacokinetics and nephrotoxicity. This likelihood seems highly probable considering size of the daily dose. Although Shibata et al.[50] stated that no nephrotoxicity was observed in mice, their study did not include interim sacrifices of animals to determine if nephrotoxicity had occurred.

3. HQ Does Not Act Like a Classical Renal Carcinogen

The USEPA[56] (1991) has recently profiled the biological characteristics of classical renal carcinogens. In **Table 10**, the biological profile for HQ is matched against these characteristics. The only match between the two profiles is that HQ is positive in non-bacterial in vitro mutagenicity assays. Classical renal carcinogens are typically also positive in bacterial systems where HQ is not active. Thus there is very little basis for considering that HQ is acting biologically like recognized renal carcinogens.

Table 10

HQ Does Not Act Like A Classical Renal Carcinogen

Classical Renal Carcinogen[1]	HQ Results
• Positive in short-term mutagenicity assays	• Negative in bacterial systems
• Induces malignant tumors	• No
• Induces high incidence of tumors	• No
• Requires minimal exposure durations	• No
• Associated with a decreased latency period	• No
• Little sex difference in susceptibility	• No

[1] USEPA Risk Assessment Forum Report, Alpha-2μ-Globulin: Association with Chemically-induced Renal Toxicity and Neoplasia in the Male Rat, EPA/625/3-91/019F, September 1991, Washington, DC.

(continued . . .)

D. Mononuclear Cell Leukemia in Female F-344 Rats Is Not a Reproducible Effect Indicative of Carcinogenicity

Table 11 includes the mononuclear cell leukemia incidence data available on HQ for female F-344 rats. The only statistically significant effect was noted at the high dose level in the NTP study. Even at the high dose level, the incidence of mononuclear cell leukemia was within the historical control limits. The incidence data for mononuclear cell leukemia in male F-344 rats and for leukemia in male and female mice in the NTP study did not differ from the respective control groups and thus do not support the premise that HQ is a leukemogen in female F-344 rats. In any case, of particular recent concern is the increasing incidence of mononuclear cell leukemia in F-344 control rats used in the NTP studies.

Table 11

Mononuclear Cell Leukemia in Female F344 Rats - 1

	NTP Historical Control[1]	NTP Bioassay (1989)			Shibata *et al.* (1991)	
		0 mg/kg	25 mg/kg	50 mg/kg	0 mg/kg	360 mg/kg
No. Tumors	75/299[2]	9/55	15/55[4]	22/55[4]	6/30	8/30
Incidence (%)	25 ± 15[3]	16	27	40	20	26

[1] NTP Water Gavaged Female F344 Rats
[2] No. Tumors/No. Animals
[3] Mean ± SD
[4] Statistically Significant Trend

(continued . . .)

The instability in the incidence of leukemia can lead to serious interpretation problems. As an example, in a recent NTP[38] (1991) bioassay on tris (2-chloroethyl) phosphate, the incidence of mononuclear cell leukemia in female F-344 rats was 14/50 or 28% (control), 16/50 or 32% (low dose), and 20/50 or 40% (high dose), results which are very similar to those seen in the NTP HQ bioassay. Yet the NTP concluded that the increases were marginal, within the historical control range, and not clearly related to administration of the test chemical.

Considering the problem that unstable incidences of mononuclear cell leukemia can have in interpretation of NTP studies, it may be significant that, in Japan where the incidence rate for thus tumor is stable, no effect on leukemia incidence was observed following HQ exposure (Shibata et al.,[50] 1991).

E. The Incidence of Hepatocellular Adenomas in B6C3F$_1$ Mice is Questionable Evidence of Carcinogenicity

Table 12 includes the incidence data for liver tumors in B56C3F mice. These data should rightfully be questioned because the incidence of adenomas in female mice: (a) did not follow a clear dose response; and (b) was close to the upper historical control range for untreated female mice.

Similarly, the data for the male mice, while consistent between the NTP[39] (1989) and the Shibata et al.[50] (1991) studies, are close to the historical incidence for these tumor types and in the NTP study actually show a reduction in malignancies. The NTP[39] (1989) interpreted the male mouse data as no evidence of carcinogenicity, while Shibata et al.[50] (1991) interpreted similar data in the opposite way.

V. Genotoxicity, Carcinogenicity and HQ Exposure

A. Introduction

HQ has been tested in a large number of assays for genotoxic effects. Many of these assays were tabulated in the NTP[39] (1989) bioassay report. **Tables 13 (a-h)** include data from the NTP report and additional studies which have been reported and reviewed since the publication of the NTP report. In general these reports fall into a number of categories including bacterial mutagenesis assays, Drosophila sex-linked

(continued . . .)

Table 12

Hepatocellular Adenomas in B6C3F$_1$ Mice

	NTP Historical Control[1]	NTP Bioassay (1989)			Shibata *et al.* (1991)	
		0 mg/kg	50 mg/kg	100 mg/kg	0 mg/kg	~1300 mg/kg
Females						
No. Tumors	22/348[2]	2/55	15/55[4]	12/55[4]	0	1/30
Incidence (%)	6 ± 5[3]	4	27	22	0	3
Males						
No. Tumors	54/347	9/55	21/55[4,5]	20/55[4,5]	6/27	14/30[4]
Incidence (%)	16 ± 4	16	39	36	22	47

[1] NTP Water Gavaged B6C3F$_1$ Mice
[2] No. Tumors/No. Animals
[3] Mean ± SD
[4] Statistically Significant for Adenomas
[5] Statistically Significant Negative Trend for Carcinomas

(continued . . .)

recessive mutation assays; in vitro chromosomal effects assays in higher plants, fungi, and mammalian cells; in vivo murine chromosomal effects assays by parenteral or oral routes of exposure, and assays for DNA adducts in vivo and in vitro.

Interpretation of these assays is not straight forward and none of the assays are designed to be directly extrapolated for risk assessment purposes. For most of these assays, correlations between the assay results may be more important than the result of any one test. For example, Ishidate et al.[24] (1981) have shown that chemicals which are positive in both the Ames/Salmonella test and in vitro CHO cell chromosomal aberration test are likely to be associated with carcinogenic activity and, conversely, chemicals which are Ames/Salmonella test negative but positive for chromosomal aberrations, are unlikely to be carcinogens.

The pattern of effects associated with HQ exposure in these studies is fairly well recognized. HQ has generally produced negative results in Ames/Salmonella and Drosophila assays, and generally positive results in chromosomal assays in vitro and in vivo when given by parenteral routes of exposure. DNA adduct formation has been positive in vitro but negative in vivo. When given orally, HQ has produced micronuclei at high dose levels but has been negative for dominant lethal effects. Thus the primary concern raised by genotoxicity screening tests have been for chromosomal damage particularly aneuploidy and related effects.

If the results of the screening assays listed in **Tables 13 (a-h)** were all that were known about HQ, they might raise serious concerns. Fortunately, there is much more information available which needs to be considered and which should also reduce the level of concern.

This information (described below) leads to two conclusions:

- The in vitro studies for HQ greatly exaggerate the risks associated with HQ exposure and should not be used as a direct assessment of human risk. (See Section B below.)

- Chromosomal effects, particularly micronuclei, from HQ at 80 mg/kg (Ciranni et al.,[9] 1988) and 200 mg/kg (Gad-El Karim et al.,[17] 1986) do not predict the NTP bioassay results in the target tissue (i.e., bone morrow) or the target species (i.e., mouse) and therefore can not be used to predict carcinogenic effects in humans or, for that matter, other species. (See Section C below.)

(continued . . .)

Table 13 a

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 1

Test System/Reference	Endpoint	Results
Bacteria		
Salmonella typhimurium		
Epler *et al.*, 1978	Gene mutation	Negative
Florin *et al.*, 1980		Negative
Rapson *et al.*, 1980		Negative
Gocke *et al.*, 1981		Positive (a)
Haworth *et al.*, 1983 (NTP)		Negative
Sakai *et al.*, 1985		Negative
Insects		
Drosophila melanogaster		
Gocke *et al.*, 1981	Sex-linked recessive lethal mutations	Negative
NTP, 1989	Sex-linked recessive lethal mutations	Negative

(a) Positive result was obtained with genetically uncharacterized strain in nonstandard medium.

(continued . . .)

Table 13 b

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 2

Test System/Reference	Endpoint	Results
Higher Plants		
Allium cepa		
Krogulevich and Stom, 1969	Chromosomal aberrations	Negative
	Chromosomal thickening	Positive
Chara zeylanica		
Chatterjee and Sharma, 1972	Chromosomal breaks	Positive
Callisia fragrans		
Roy, 1973	Polyploidy	Negative
Fungi		
Aspergillus nidulans		
Crebelli *et al.*, 1987 and 1991	Chromosomal aberrations	Positive
Kappas, 1990	Mitotic cross-overs	Positive
Strain D6		
Parry *et al.*, 1990	Induced chromosome loss	Positive

(continued . . .)

Table 13 c

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 3

Test System/Reference	Endpoint	Results
Mammalian cells (*in vitro*)		
Mouse lymphoma cells		
Pellack-Walker and Blumer, 1986	DNA strand breaks	Negative
Pellack-Walker *et al.*, 1985	Inhibition of DNA synthesis	Positive
McGregor *et al.* 1988 (NTP)	Trifluorothymidine resistance	Positive
Mouse bone marrow cells		
Lee *et al.*, 1989	DNA synthetic activity	Positive
Chinese hamster ovary cells		
Galloway *et al.*, 1987 (NTP)	Sister chromatid exchanges	Positive
	Chromosomal aberrations	Positive
Chinese hamster Don cells		
Shimada *et al.*, 1988	Sister chromatid exchanges	Positive

(continued . . .)

Table 13 d

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 4

Test System/Reference	Endpoint	Results
Mammalian cells (*in vitro*) cont.		
Human HeLa cells		
Painter and Howard, 1982	Inhibition of DNA synthesis	Positive
Human lymphocytes		
Morimoto *et al.*, 1983	Sister chromatid exchanges	Positive
Knadle, 1985		Positive
Rat Zymbal Gland Cells		
Reddy *et al.*, 1990	DNA adducts	Positive
Human Promyelocyte Leukemia (HL-60) Cells		
Levay *et al.*, 1991	DNA adducts (b)	Positive
CHO cells		
Parry *et al.*, 1990	Micronuclei	Negative
	Mitotic division aberrations	Positive

(b) HL-60 adducts and calf thymus DNA adducts differ.

(continued . . .)

Table 13 e

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 5

Test System/Reference	Endpoint	Results	Exposure Route
Mammalian cells (*in vivo*)			
Mice (DDY)			
Shimada *et al.*, 1988	Micronuclei	Positive	NS
	DNA strand breaks	Positive	NS
Mice (NMRI)			
Tunek, 1982	Micronuclei	Positive	SC
Mice (102/E1 x C3H/E1)F^1			
Xu and Adler, 1990	Micronuclei	Positive	NS
	Chromosomal aberrations	Positive	NS
Mice (NMRI)			
Gocke *et al.*, 1981	Micronuclei	Positive	IP
Mice (C57BL)			
Gocke *et al.*, 1983	Gene mutation	Negative	IP
Mice (101/E1 x C3H/E1)F_1			
Adler and Kliesch, 1990	Micronuclei	Positive	IP

NS - Route not specified.

(continued . . .)

Table 13 f

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 6

Test System/Reference	Endpoint	Results	Exposure Route
Mammalian cells (*in vivo*) cont.			
Mice (Swiss CD-1)			
Barale *et al.*, 1990	Micronuclei	Positive	IP
Mice (Swiss CD-1)			
Ciranni *et al.*, 1988	Micronuclei	Positive	IP
Mice (CBA)			
Jenssen and Ramel, 1980	Micronuclei	Positive	IP
Mice (102/E1 x C3H/E1)F_1			
Ciranni and Adler, 1991	Chromosomal aberrations (male germ cells)	Positive	IP
Mice (C57Bl/Cne x C3H/Cne)F_1			
Pacchierotti *et al.*, 1991	Micronuclei	Positive	IP
	Cell-cyle lengthening	Positive	IP
	Hyperploidy	Positive	IP
	Sister chromatid exchange	Negative	IP

(continued . . .)

Table 13 g

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 7

Test System/Reference	Endpoint	Results	Exposure Route
Mammalian cells (*in vivo*) cont.			
Mice (102/E1 x C3H/E1)F_1			
Xu and Adler, 1990	Chromosomal aberrations	Positive	IP
Mice (101/E1 x C3H/E1)F_1			
Miller and Adler, 1989	c-Mitotic effects	Positive	IP
Mice			
Marrazzini *et al.*, 1991	Micronuclei	Positive	IP?
	Chromosomal aberrations	Positive	IP?
	Aneuploidy	Positive	IP?
	Polyploidy	Positive	IP?
Mice (CD-1)			
Gad-El-Karim *et al.*, 1986	Micronuclei	Positive	PO
Mice (Swiss CD-1)			
Ciranni *et al.*, 1988	Micronuclei	Positive	PO

(continued . . .)

Table 13 h

Summary of Results of Genetic Toxicology Studies of Hydroquinone - 8

Test System/Reference	Endpoint	Results	Exposure Route
Mammalian cells (*in vivo*) cont.			
Rat (S-D)			
Krasavage, 1984	Dominant Lethal	Negative	PO
Rat (S-D)			
Reddy, 1990	DNA Adducts (c)	Negative	PO

(c) Bone marrow, Zymbal gland, liver and spleen were studied using P1-enhanced ^{32}P postlabeling.

(continued . . .)

B. The *In Vitro* Studies for HQ Greatly Exaggerate the Risks Associated With HQ Exposure and Can Not Be Used For Risk Assessment Purposes

One major problem with in vitro assays of HQ is that the relative water solubility of HQ allows exposure of target tissues at much higher concentrations in vitro than can be achieved during in vivo testing. This is a problem associated with in vitro assays of many chemicals and is not unique to HQ studies.

A larger problem is that the in vitro studies of HQ are essentially irrelevant for risk assessment purposes because the reduction oxidation potentials in the culture systems were uncontrolled. Greenlee et al.[21] (1981) have shown that HQ in a phosphate buffer (pH 7.4) exposed to air autoxidizes to p-benzoquinone. At a concentration of 0.4mM, HQ autoxidizes to p-benzoquinone at a rate of 4.8 $\pm$ 0.3 nmol/min. In a similar fashion, Irons and Neptun[23] (1980) showed that hydroquinone inhibition of microtubule polymerization, and acceleration of the decay of tubulin-colchicine binding activity did not occur if HQ autoxidation was prevented.

Because cell culture systems are artificial systems lacking the protective mechanisms present in the whole organism, it is not possible to make a direct extrapolation between the cell culture conditions and the in vivo situation.

The importance of cellular and whole organism homeostatic mechanisms is most clearly demonstrated by contrasting the DNA adducts measured by ^{32}P-postlabeling using isolated calf thymus DNA, HL-60 cells and intact rats. In isolated DNA and HL-60 cultures, Levay et al.[32] (1991) were able to detect HQ-DNA adducts but the type of adduct differed in the two systems suggesting the cellular mechanisms modified the HQ-DNA interaction. In contrast to these results, Reddy et al.[47] (1990) found no HQ-DNA adducts in the liver, spleen, bone morrow, and Zymbal glands of intact rats.

C. Chromosomal Effects, Particularly Micronuclei, Did Not Predict the Bioassay Results with HQ

The largest number of in vitro and in vivo assays with HQ have studied chromosomal damage, particularly using red blood cell micronuclei as a significant endpoint. For example, doses of 80 mg/kg (Ciranni et al.,[9] 1988) and 200 mg/kg (Gad-El Karim et al.,[17] 1986), HQ produced micronuclei in CD-1 mice. Based on these findings, it is reasonable to expect that chronic oral HQ exposure should induce hematopoietic tumors in mice. Yet the male and female mice in the study by Shibata et al.[50] (1991)

(continued . . .)

and the male mice in the NTP study showed no increase in hematopoietic tumors at approximately 1300 mg/kg/day and 50 and 100 mg/kg/day, respectively. The female mice in the NTP study actually showed a statistically significant trend towards a reduction in hematopoietic tumors. As a result, in the target species (mouse) and tissue (bone marrow), clastogenicity did not correlate with tumorigenicity.

D. Summary:

In summary, then, the following points can be made in relationship to a qualitative assessment of the in vitro data on HQ:

- HQ is not genotoxic in bacterial assays.
- In vitro cell assay results are not relevant for predicting genotoxicity for HQ.
- In vivo assays for genotoxicity are negative except at high dose levels of HQ when exposure is by a relevant route.
- In vivo clastogenicity following HQ exposure is not relevant to predicting animal or human tumorigenicity.

VI. Research Work Underway or Planned

An in-depth Research Program to further the understanding of HQ toxicity is underway or planned. The Research program is outlined in **Figure 2**.

These studies are directed toward developing a nephrotoxicity model system in rats to better understanding of species differences in HQ metabolism. This work has demonstrated that nephrotoxicity can be produced in F-344 rats with a single oral dose of HQ and that Sprague-Dawley rats do not develop nephrotoxicity under similar conditions (i.e., oral dosing). Development of this system has already allowed identification of a nephrotoxic HQ metabolite that can induce nephrotoxicity in F-344 and Sprague-Dawley rats. Preliminary results of this work were presented at the 1992 Society of Toxicology meeting. Studies to measure cell proliferation in the kidneys of F-344 rats given HQ are also underway but final results are not available at this time. The aim of this program is to develop an integrated model for HQ toxicity which includes a biological model component and a physiologically-based pharmacokinetics model component.

(Figure 2 and Tables 14 and 15 follow.)

(continued . . .)

Figure 2: Timeline for Research Activities on Hydroquinone

Jan. 1992	Pharmacokinetic study completed. Kidney cell proliferation study (oral dosing) underway.
Feb. 1992	Presentation to Society of Toxicology on acute-dose model system for study of HQ nephrotoxicity.
March 1992	^{32}P-postlabeling study in rats (oral dose) underway.
May 1992	Oral-dose cell proliferation study in one-year-old rats started. Meet with FDA to discuss research plans. Decision on specific vehicle for dermal studies.
June 1992	Initiation of 14-day dose-ranging study for 13-week dermal study in rats. Initiation of Dermal Absorption/Distribution/Metabolism/Excretion Study.
June 1992	Oral-dose cell proliferation study in young rats completed.
July 1992	^{32}P-postlabeling study completed.
Nov. 1992	14-day dermal study in rats completed. 13-week dermal study, including measurement of kidney cell proliferation, underway.
Jan. 1993	In-life phase of 13-week study in rats completed.
Feb. 1993	Cell proliferation study in one-year-old rats completed.
April 1993	Full Toxicity Review
June 1993	Two-year dermal bioassay, pending Toxicity Review

Table 14

Summary of HQ Metabolism in a Human Volunteer, F344 Rats, SD Rats and Mice*

	SPECIES								
	HUMAN n = 1	F344 RAT n = 4		SD RAT n = 2		MOUSE n = 3		F344 RAT 15 day n = 4	
SEX	M	M	F	M	F	M	F	M	F
METABOLITE	PERCENT OF TOTAL RECOVERED IN URINE								
HQ-Glucuronate	54.8	61.9±3.7	62.0±6.2	59.7±1.4	75.0±2.2	85.6±0.4	75.3±0.6	56.2±4.8	63.7±9.5
HQ-Sulfate	38.0	35.8±2.7	29.9±2.8	38.6±0.5	20.0±2.2	14.4±0.4	24.7±0.6	41.3±2.2	26.9±3.8
HQ-Mercap	7.1	<loq§	3.6±2.8	2.1±0.9	5.0±0.0	<loq	<loq	0.2±0.1	0.9±0.9
HQ	0.01	1.6±0.8	3.8±3.6	<loq	<loq	<loq	<loq	2.0±1.3	8.5±8.3

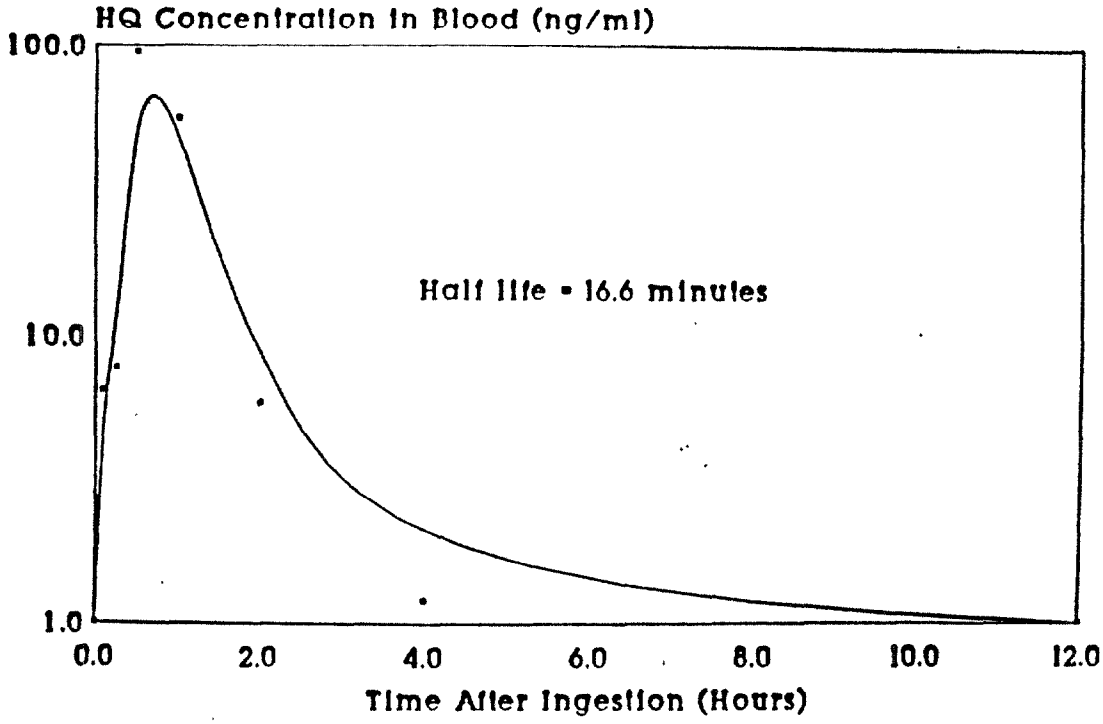
* The human dose was 275 mg or 4 mg/kg and the rodent doses were 25 mg/kg. These doses are roughly equivalent with respect to body surface area.

§ <loq = mean values were less than the lower limit of quantitation.

(continued . . .)

Table 15

Kinetics of Oral Hydroquinone (275 mg) in a Male Volunteer



(continued . . .)

Pharmacokinetics studies with HQ were completed as part of the USEPA TSCA Section 4 test rule. Additional data on the comparative metabolism of HQ in different species is presented in **Table 14** and the blood kinetics of HQ in a human volunteer is presented in **Table 15**. A proposed metabolic pathways for HQ biotransformation in F-344 rats is presented in **Figure 3**.

Figure 3 shows that significant conjugation of hydroquinone to its glucuronide and sulfate metabolites occurs in the intestinal tract where large amounts of high capacity conjugation enzymes are located (Cassidy and Houston,[72] 1984). Hydroquinone which escapes conjugation in the intestinal tract is available for absorption and can be transported to the liver where further sulfate, glucuronide, or glutathione conjugation can occur. The sulfate and glucuronide metabolites are considered detoxification products and can be readily excreted in the urine. Hydroquinone can also potentially be metabolized through a series of reactions to mono-, and di-, or tri-glutathione conjugates which are excreted into the bile and reabsorbed from the intestine as cysteine conjugates. The cysteine conjugates can be metabolized in the liver or more probably in the proximal renal tubular epithelium to a N-acetyl cysteine conjugate of hydroquinone. The cysteine and N-acetyl cysteine conjugates may accumulate in the renal epithelium because they move into the cells by transport systems, while they leave the epithelium only by diffusion. The metabolic cycling between these two materials may lead to oxidative stress in the renal epithelial cells and toxicity in the F-344 rat.

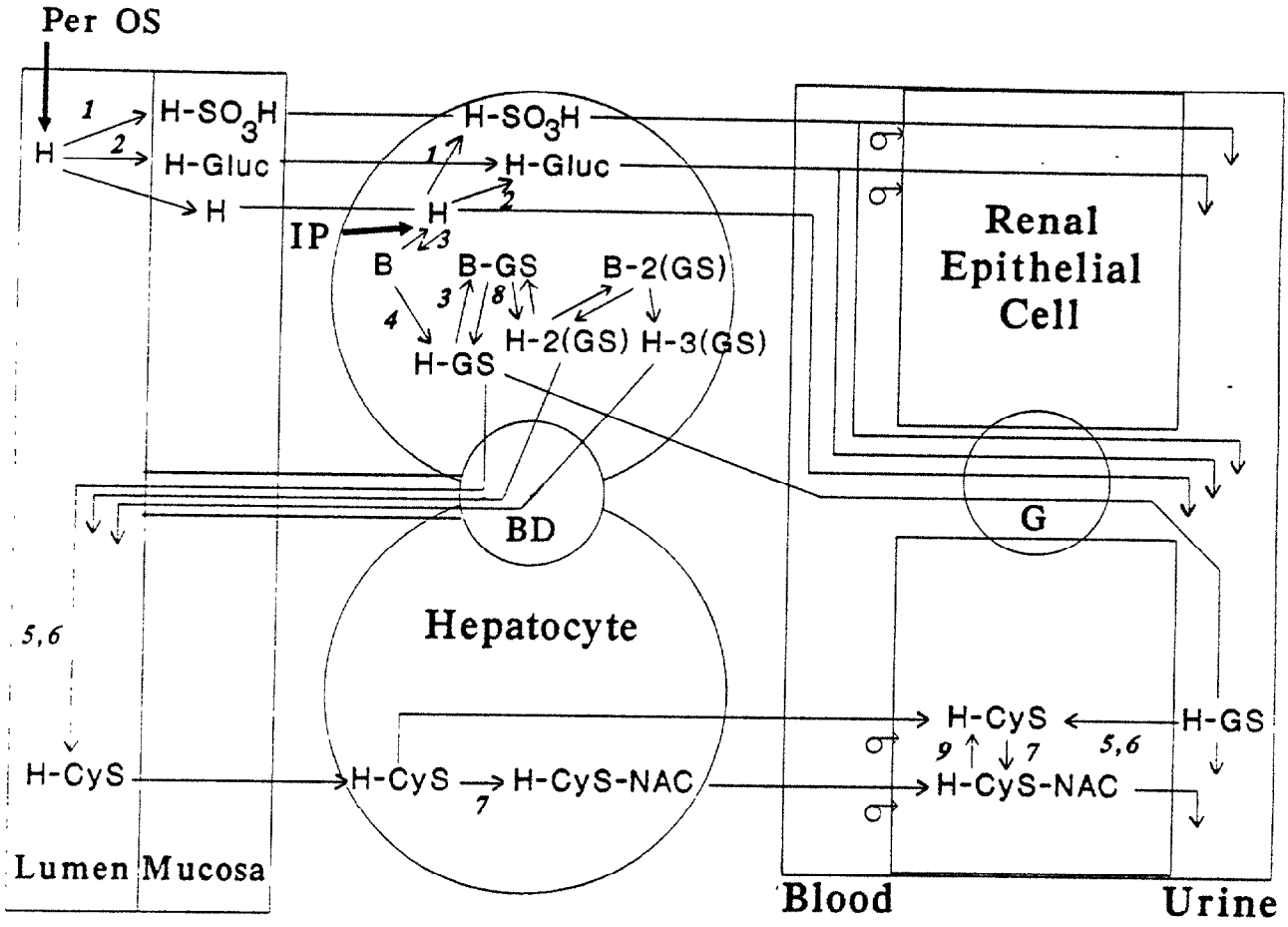
All of this work to date has been conducted using oral HQ exposures. With the low degree of penetration seen after HQ application to the skin and because clearance mechanisms rapidly remove HQ from the blood, the risks associated with dermal exposure to HQ should be extremely small even for the F-344 rat. To further characterize this issue, NDMA's Hydroquinone Task Group plans to implement a long term dermal bioassay according to the draft protocol provided in the references (see NDMA,[40, 41] 1991). The Task Group is in the end stages of completing this protocol in order to initiate the studies in the very near future (see **Figure 2**). NDMA would estimate at this time that the completion of the dermal bioassay would take approximately 2.5 to 3 years.

In summary, based on the data available, we have generated a hypothesis about how and why HQ exposure results in renal tumors in male F-344 rats. When HQ is given orally to the F-344 rat, it is rapidly absorbed and rapidly metabolized in the liver to sulfate, glucuronide, and glutathione conjugates. The glutathione conjugate and/or its metabolites are mildly toxic to the renal tubular epithelium which undergoes regenerative hyperplasia or cell proliferation. The susceptibility to nephrotoxicity can be transferred to Sprague-Dawley rats by intravenous injection of the HQ-glutathione conjugate. Both male and female F-344 rats are susceptible

(continued . . .)

Figure 3

Proposed Site-Specific Metabolism For Hydroquinone



Intestine Liver Kidney

Legend for Figure 3

1	Sulfuryl Transferase Catalyzed
2	Glucuronide Transferase Catalyzed
3	P-450 Catalyzed
4	GST Catalyzed
5	γ-GT Catalyzed
6	Peptidase Catalyzed
7	N-acelyltransferase Catalyzed
8	Quinone Reductase Catalyzed
9	Deacetylase Catalyzed
H	Hydroquinone
B	Benzoquinone
G	Renal Glomerulus
⊖→	Transport System
BD	Bile Duct
B-GS	Benzoquinone Glutathione Conjugate
B-2(GS)	Benzoquinone Diglutathione Conjugate
H-Gluc	Hydroquinone Glucuronide
H-GS	Hydroquinone Glutathione Conjugate
H-2(GS)	Hydroquinone Diglutathione Conjugate
H-3(GS)	Hydroquinone Triglutathione Conjugate
H-CyS-NAC	Hydroquinone N-Acetyl Cysteine Conjugate
H-CyS	Hydroquinone Cysteine Conjugate
$H\text{-}SO_3H$	Hydroquinone Sulfate Conjugate
Per OS	Oral Administration of Hydroquinone
IP	Intraperitoneal Administration of Hydroquinone

to HQ renal toxicity, but the males differ from the females in two ways. The first is that the response of the females is less severe than that of the males when the animals are exposed to HQ chronically or subchronically. The second is that, in the males, spontaneous chronic progressive nephropathy is much more serious than in females. These two phenomena are likely to lead to higher levels of chronic cell proliferation in males than in females. The consequence of this combined stimulus to cell proliferation would be expected to result in an increased risk of tumorigenicity for male F-344 rats that would not be shared by female F-344 rats or other strains of rats and species of animals which do not develop nephrotoxicity following HQ exposure.

VII. Conclusion

Therefore, given that foregoing commentary about the available data, NDMA's Hydroquinone Task Group concludes that hydroquinone in OTC skin lightening preparations is not likely to represent a human risk when used according to label directions.

Respectfully submitted on behalf of the NDMA Hydroquinone Task Group,

R. William Soller, Ph.D.
Senior Vice President
Director of Science & Technology
Nonprescription Drug Manufacturers Association

(Reference List follows; Tables follow references.)

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(continued . . .)

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